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Integrating bioengineered F1 motors into nano-structured surfaces

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5d. PROJECT NUMBER

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14. ABSTRACT

The project is focused on the remarkable F1 rotary motor protein that couples the hydrolysis of ATP to unidirectional rotation of a centrally located protein element (spindle). The main object is to fabricate a platform nano-device that couples rotation of the spindle element to drive a secondary device such as a propeller or a switch to produce a desired response. The F1 motor must be in a fixed location and orientation on a solid surface to interface with the secondary device. In this study the secondary device is a nano-electrode in which electrical

15. SUBJECT TERMS

Engineered F1 motors surface attachment nano-electrode fabrication

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a. REPORT	b. ABSTRACT	c. THIS PAGE			Mark Richter
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Report Title

Integrating bioengineered F1 motors into nano-structured surfaces

ABSTRACT

The project is focused on the remarkable F1 rotary motor protein that couples the hydrolysis of ATP to unidirectional rotation of a centrally located protein element (spindle). The main object is to fabricate a platform nano-device that couples rotation of the spindle element to drive a secondary device such as a propeller or a switch to produce a desired response. The F1 motor must be in a fixed location and orientation on a solid surface to interface with the secondary device. In this study the secondary device is a nano-electrode in which electrical current is produced as a magnetic particle affixed to the rotating spindle rotates across it. Protein engineering introduced a special armature within the rotating spindle element of the enzyme extending the diameter of rotation of attached objects beyond the periphery of the motor protein. Atomic force microscopy and nanografting methods were used to etch nano-electrodes into material surfaces for enzyme attachment, and specialized surface attachment methods developed to attach F1 motors to defined regions on chemically modified solid surfaces in correct orientations. Current studies aim at integrating the engineered F1 motors into the etched nano-electrodes to demonstrate interconversion of electrical and chemical (ATP) energy.

Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

Received	Paper

TOTAL:

Number of Papers published in peer-reviewed journals:

(b) Papers published in non-peer-reviewed journals (N/A for none)

Received	Paper

TOTAL:

Number of Papers published in non peer-reviewed journals:

(c) Presentations

1. Richter, M.L., Berrie, C.L. & Gao, F. Engineering of the F1 ATPase rotary motor, Invited talk at the Chinese National Academy of Sciences, Beijing, August 27, 2010

2. J.K. Settle, M.L. Richter, C.L. Berrie, Towards F1-ATPsynthase Based Hybrid Nanobiodevice Fabrication, International Meeting of the American Vacuum Society, Nashville, TN, Oct. 30-Nov 4, 2011.

3. Smith, G.; Berrie, C. L.; Nanoscale Surface Patterning for Controllable Metal Depostition, International Meeting of the American Vacuum Society, Nashville, TN, Oct. 30-Nov 4, 2011.

Number of Presentations: 3.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received	Paper	
12/27/2011	2.00	Stephanie Bishop, Kim Colvert, Daxin Zheng, Mark Richter, Cindy Berrie, Fei Gao. Insertion of a Rigid Structural Element into the Regulatory Domain of the Chloroplast F1-ATPase Gamma Subunit for Rotational Studies., 15th International Photosynthesis Congress. 2010/08/22 01:00:00, . : ,
12/27/2011	3.00	. The Mutation E242K in the chloroplast ATP synthase Gamma Subunit Increases the Inhibitory Binding of the Epsilon Subunit Without Changing the Apparent Redox Potential of the Regulatory , 15th International Photosynthesis Congress. 2010/08/22 01:00:00, . : ,
TOTAL:	**2**	

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received	Paper	
TOTAL:		

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts):

(d) Manuscripts

Received	Paper

TOTAL:

Number of Manuscripts:

Books

Received	Paper

TOTAL:

Patents Submitted

Patents Awarded

Awards

Graduate Students

NAME	PERCENT_SUPPORTED	Discipline
Jennifer Settle	0.50	
Greg Smith	0.10	
FTE Equivalent:	**0.60**	
Total Number:	**2**	

Names of Post Doctorates

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Names of Faculty Supported

NAME	PERCENT_SUPPORTED	National Academy Member
Cindy Berrie	0.08	
Mark Richter	0.04	
FTE Equivalent:	**0.12**	
Total Number:	**2**	

Names of Under Graduate students supported

NAME	PERCENT_SUPPORTED	Discipline
Shyam Mehta	0.10	Biochemistry
Alan Shi	0.00	Chemistry
Brittney Ridl	0.00	Chemistry
Aimee Bigger	0.10	Biochemistryn
FTE Equivalent:	**0.20**	
Total Number:	**4**	

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period:	1.00
The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:.....	1.00
The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:.....	1.00
Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):	1.00
Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:	0.00
The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense	1.00
The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields:	1.00

Names of Personnel receiving masters degrees

NAME
Total Number:

Names of personnel receiving PHDs

NAME
Total Number:

Names of other research staff

NAME	PERCENT_SUPPORTED
Kim Colvert	0.15
Denise Mills	0.33
FTE Equivalent:	**0.48**
Total Number:	**2**

Sub Contractors (DD882)

Inventions (DD882)

Scientific Progress

(1). Forward. This is a collaborative interdisciplinary project to establish technology for integrating complex protein structures into inorganic material substrates for fabrication of nanocomposite materials and devices for a broad range of applications. Previous funding resulted in engineering of F1 motor proteins with specialized features for surface attachment in addition to extensive studies of the rotational mechanism and development of analytical methods for orientational protein patterning. The specific target of the current project is the on-chip fabrication of a hybrid organic/inorganic nanodevice that couples the rotation of a spindle element of the F1-ATPase motor to a secondary device such as a nanoscale electricity generator or a nano-scale pump. The considerable technical challenges associated with this task include implementation of protein engineering strategies to introduce specialized features into the F1 molecule, development of new methods for precise attachment of the motor into patterned surface features in correctly oriented functional form, and creation of novel surface secondary devices for translation of the ATP-driven rotation of the F1 spindle element into useful work.

(2). Statement of the problem studied. The work to date has focused on fabrication of a simple device for reversible conversion of rotational energy produced via the chemical breakdown of adenosine triphosphate (ATP) into electrical current. Such a device would consist of the F1 motor placed in a confined feature (box or cylinder) etched into a silicate-coated surface surrounded by one or more nano-electrodes. Rotation of a magnetic bead attached to an armature that has been engineered into or chemically attached to the central rotating spindle element of the F1 motor must pass over the electrode to create measurable (nW) current. This work, although proving extraordinarily challenging, has resulted in success on several fronts as indicated below.

(3). Summary of the most important results.
(3.a). Protein engineering. Two engineered protein constructs have been prepared in the Richter lab. In each case a small, rigid protein extension has been genetically incorporated into the rotating spindle in a position expected to extend the spindle at 90o to the central rotating axis of the motor. In one case, the twisted helical repressor of plasmid protein (Rop) was incorporated producing gamma-Rop. In the second case the rigid beta barrel green fluorescent protein (GFP) was incorporated producing gamma-GFP. Remarkably, both engineered constructs were effectively assembled with other motor protein subunits to produce highly active motors. Detailed biochemical analysis of the two constructs has been completed. The last step involved engineering of binding sites in the two constructs for attachment of magnetic particles. In the case of gamma-Rop, a cysteine residue has been engineered into the loop connecting the two helices of the Rop extension for particle attachment via a biotin-maleimide adduct [1]. In addition, three cysteines, two in the Rop protein and one in the rotating spindle element (the gamma subunit) had to be removed. This has now been achieved but caused considerable difficulty this past year – attempts to introduce multiple mutations simultaneously within the construct were unsuccessful necessitating the introduction of each mutation individually. In spite of this problem, we now have the complete gamma construct as indicated in Figure 3 which assembles with the alpha and beta subunits to produce a fully functional enzyme as shown by the data of Figure 4. In the case of gamma-GFP, an avidin binding site has been engineered into the outwardly extended end of the beta barrel structure for attachment of metallic beads via an avidin-biotin connector [2].

The two engineered proteins are ready for surface attachment which will be followed by attachment of metallic beads for extensive rotational studies and for integration into the nano-electrode system. Preliminary rotation studies have been performed with the hybrid enzyme and we have been able to demonstrate rotation of gold beads attached to non-engineered enzyme according to previously published methods [2]. However, specific attachment of the gold particle on the rotating spindle armature has not been possible due to the presence of exposed cysteinyl side chains on the alpha subunit that compete for binding of the functionalized nanoparticles. This problem is confounded by the fact that there are three alpha subunits per molecule leading to multiple attachment sites and considerable heterogeneity of labeled molecules, most of which fail to rotate since the alpha subunits are static. Therefore, an additional cloning step has been undertaken to identify and remove the exposed cysteines. The final construct is expected to be ready for assembly and rotation studies within the next few weeks.

Work has also been in progress to determine the structural basis of a dithiol “switch” located in the regulatory domain within the rotating gamma subunit of the F1 motor. Significant insight into the mechanism of regulation of rotation has been obtained through a combination of site-directed mutagenesis and structural modeling studies. The information will be used for future studies in which ligand binding protein sequences will be introduced within the domain to induce ligand-dependent modulation of rotation – that is a ligand-induced switch that either activates or inactivates rotation. This work has been performed by Dr. Kim Colvert, a visiting scientist while on sabbatical in the lab. A preliminary publication was included in the Proceedings of the International Photosynthesis Congress in 2010 and another publication describing an extension of this work is in preparation for submission.

(3.b). Surface Attachment Strategies. In order to create the hybrid nanodevices envisioned in this work, the protein must be attached to the surface in a functionally active way and oriented and aligned with electrodes incorporated into the design of the structure. All of this must be accomplished on the nanometer length scale. We have employed AFM based methods for generating patterns of both the protein and the metal electrodes on the surface. First, we attach the protein to the surface selectively in nanometer scale functionalized regions activated with an NTA group to promote specific binding of the protein tag

on the pattern. This is accomplished through a two step grafting process in which a dithiol molecule is patterned into the surface and then the dithiol molecule is reacted with a maleimide functionalized NTA in order to introduce the NTA functionality locally on the surface only in the patterned regions. The protein can then be selectively attached to the NTA groups through engineered histidine tags. The electrodes (to detect or drive protein rotation) are fabricated using electroless deposition of copper either on silicon or gold surfaces. On the gold surface. The electrodes can be introduced by patterning a carboxylic acid group into the dodecane thiol matrix and allowing electroless deposition to occur selectively on the patterned region of the surface. The patterning on silicon is accomplished by selectively removing a monolayer resist from the silane coated silicon surface and allowing deposition to occur only in the exposed regions. In order to create the nanopatterned devices, both of these structures must be deposited on the nanometer length scale with very precise spatial registry. This is accomplished using the nanografting approach which allows nanometer scale control over the motion of the probe tip as well as patterning of multiple materials sequentially in a fluid cell.

During the most recent period, we have demonstrated the ability to selectively functionalize the gold surface with the NTA functional group required for specific immobilization of the F1 ATPase on the surface. Coordination of nickel ions and the histidine tags on the protein have also been demonstrated. Figure 1 shows that the maleimide coupling chemistry to introduce the NTA groups on the surface is selective on the thiol terminated monolayer surface. No reaction with a fluorescent maleimide dye is observed on the control sample, while strong fluorescence is observed on the thiol terminated sample. The selective introduction of the thiol functional group in the matrix methyl-terminated monolayer using nanografting is demonstrated in Figure 2. The AFM created patterns were used to bind a fluorescently labeled dye, and the dye is observed in the same pattern as that of the thiol groups on the surface. These results demonstrate that the incorporation of the NTA groups on the surface in nanometer scale patterns has been achieved.

The next step is to demonstrate that the NTA groups on the surface specifically bind the F1 ATPase. This has been successfully demonstrated through both SPR measurements of protein binding and the as well as through imaging of the fluorescently labeled F1 ATPase binding to both NTA functionalizes films and the methyl terminated matrix film as a control (Figure 3). The fluorescence occurs only on the NTA terminated film and not on the methyl terminated control, indicating specific attachment of the ATPase motor to the NTA groups on the surface. SPR measurements have indicated that there is more than one rate constant for binding of the histidine tagged ATPase to the NTA terminated surface. Kinetic fits to the SPR data with a single 1:1 Langmuir adsorption model show poor fits, whereas inclusion of multiple binding pathways allows a much better fit (Figure 4). This indicates that multiple histidine groups are involved in the binding to the surface (resulting in different rate constants when different numbers of histidine tags are bound). This result indicates that multivalency is important in strongly binding the ATPase motor to the surface.

In addition to demonstrating that the F1 ATPase motors can be successfully patterned into nanometer scale domains on the surface, progress has also continued on the preparation of the metal nanowires that are to be used to detect the motion of the F1 motor. The patterns can be created using metal coated AFM probe tips to anodically oxidize patterns on the surface. Figure 5 shows that copper can be successfully patterned into nanometer scale patterns on a silicon substrate, providing the control needed to form the wires with the correct size and spatial relationship to the F1 motor. A manuscript describing this work is in the late stages of preparation for submission.

These preliminary data clearly demonstrate the feasibility that all of the components of the proposed F1 motor protein device can be successfully fabricated on the required length scales. The incorporation of each step into a single chip is a logical extension of the results obtained to date. This work is still in progress.

(4). Continuing studies.

As a result of the preliminary work we are uniquely poised to fabricate the first integrated system in which the F1 enzyme is attached to a discrete site on a material surface such that rotation of a magnetic particle attached to the rotating armature of the enzyme will pass across a nano-electode etched into the surface to produce an electrical current. This work has paved the way for extensive protein and material engineering studies to develop functioning nanodevices in which ligand-controlled rotation of the spindle element of the F1 motor will produce a measured electrical response. To date the work has been carried out as a very effective collaboration between Dr. Mark Richter, a protein chemist and enzymologist, and Dr. Cindy Berrie, a materials chemist with considerable experience in surface chemical modification and atomic force microscopy (AFM). A third collaborator, Dr. Judy Wu, has been recruited to the project. Dr. Wu is a physicist with extensive experience in fabrication of micro- and nanocircuitry on microchip surfaces and will assist in constructing patterned electrode systems into which molecular motors are integrated. Future work will aim at:

a. Development of a patterned electrode platform with integrated F1 motors. Nanoelectrodes will be fabricated on microchip surfaces by combining an AFM etching and nanografting approach with a carbon-based nanopatterning approach. F1 motors will be integrated into chemically functionalized surface patterns to produce a prototype nanodevice that interconverts chemical and electrical energy in a ligand-dependent manner;

b. Engineering of molecular recognition “switch” elements into the F1 motor for ligand-induced modulation of rotation.

Molecular modeling studies will be applied to design and engineer specific ligand binding elements into a previously identified regulatory protein domain within the rotation spindle element such that binding of a specific ligand will induce or block rotation;

c. Analysis of the rotational dynamics of the engineered F1 motor in the integrated system. Rotation of gold and magnetic particles attached to the rotating arm of the engineered F1 motor will be examined using confocal microscopy and electrical current generation respectively to determine the kinetic behavior of the rotating spindle. These studies will identify the protein-protein interactions responsible for development of rotational torque and for directional rotation of the spindle element.

(5). Literature Cited

1. Tucker, W.C., Schwarz, A., Levine, T., Du, Z., Gromet-Elhanan, Z., Richter, M.L. and Haran, G. “Observation of calcium-dependent unidirectional rotational motion in recombinant photosynthetic F1-ATPase molecules.” J.Biol.Chem. 2004, 279, 47415-47418.
2. Yasuda, R., Noji, H., Yoshida, M., Kinosita Jr. K., & Itoh, H. “Resolution of distinct rotational substeps by submillisecond kinetic analysis of F1-ATPase.” Nature 2001, 410, 898-904.

(6) Resulting Papers and Presentations

1. Bishop, S.C., Mehta, S., Colvert, K.K., Zheng, D., Richter, M.L., Berrie, C.L. & Gao, F. Insertion of a rigid structural element into the regulatory domain of the chloroplast F1-ATPase gamma subunit for rotational studies. Proceedings of the 15th International Congress on Photosynthesis, 2011, pp.123-126.
2. Colvert,K.K., Gao, F., Zheng, D., Mehta, S. & Richter, M.L. The Mutation E242K in the chloroplast ATP synthase Gamma Subunit Increases the Inhibitory Binding of the Epsilon Subunit Without Changing the Apparent Redox Potential of the Regulatory Dithiol. Proceedings of the 15th International Congress on Photosynthesis, 2011, pp.127-130.
3. Settle, J.K., Smith, G.J., Zheng, D., Richter, M.L. & Berrie, C.L. Atomic force microscopy investigation of F1 ATP Synthase on mica surfaces: Observation of multiple protein morphologies. in preparation
4. Colvert, K.K. & Richter, M.L. Mutations in the vicinity of the regulatory dithiol domain of the chloroplast F1 gamma subunit alter access of the dithiol to thioredoxin. in preparation
5. Smith, G.J. & Berrie, C. L. In Towards copper nanostructure formation using SAMs on gold and silicon substrates, American Chemical Society: 2010; pp MWRM-134, Midwest Regional Meeting of the American Chemical Society, Wichita, KS, United States, October 27-30
6. Settle, J.K., Richter, M.L. & Berrie, C.L. In Atomic force microscopy investigations of surface patterning and F1-ATPsynthase immobilization: towards hybrid nanobiodevice design, American Chemical Society: 2010; pp MWRM-212, 45th Midwest Regional Meeting of the American Chemical Society, Wichita, KS, United States, October 27-30
7. Colvert, K.K., Gao, F., Zheng, D., Mehta, S. & Richter, M.L. The Mutation E242K in the gamma subunit of the chloroplast ATP synthase increases both the binding affinity of the epsilon subunit and the apparent redox potential of the regulatory dithiol, International Congress on Photosynthesis, 2010, Proc. p.75, Beijing, August 22-27
8. Bishop, S.C., Mehta, S., Colvert, K.K., Zheng, D., Berrie, C.L., Richter, M.L., & Gao, F. Insertion of a rigid structural element into the regulatory domain of the chloroplast F1-ATPase gamma subunit for rotational studies, International Congress on Photosynthesis, 2010, proc. P193, Beijing, August 22-27
9. Richter, M.L., Berrie, C.L. & Gao, F. Engineering of the F1 ATPase rotary motor, Invited talk at the Chinese National Academy of Sciences, Beijing, August 27, 2010
10. Settle, J. K., Berrie, C. L. & Richter, M. L. In Towards hybrid nanobiodevice construction: F1-ATP synthase adsorption studies, American Chemical Society: 2011; pp MWGL-503, Joint 46th Midwest and 39th Great Lakes Regional Meeting of the American Chemical Society, St. Louis, MO, United States, October 19-22, 2011.
11. Settle, J.K., Richter, M.L. & Berrie, C.L. Towards F1-ATPsynthase Based Hybrid Nanobiodevice Fabrication, International Meeting of the American Vacuum Society, Nashville, TN, Oct. 30-Nov 4, 2011.
12. Smith, G., & Berrie, C. L. Nanoscale Surface Patterning for Controllable Metal Depostition, International Meeting of the American Vacuum Society, Nashville, TN, Oct. 30-Nov 4, 2011.

Technology Transfer

Richter/Berrie Figures

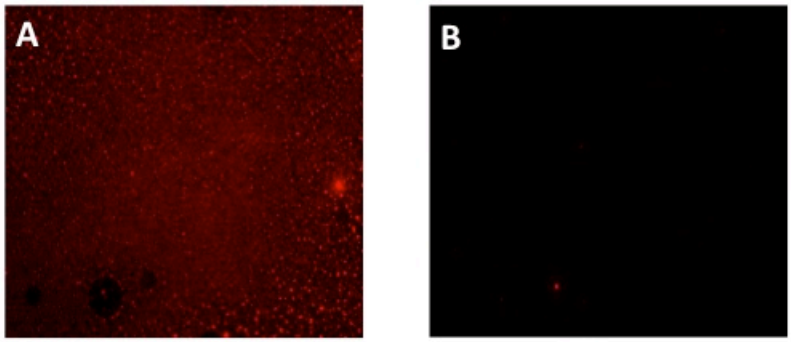

Figure 1: Control Experiment showing that the maleimide dye attaches specifically to the monolayer film terminated in thiol functional groups (A) and not on the methyl terminated control surface (B).

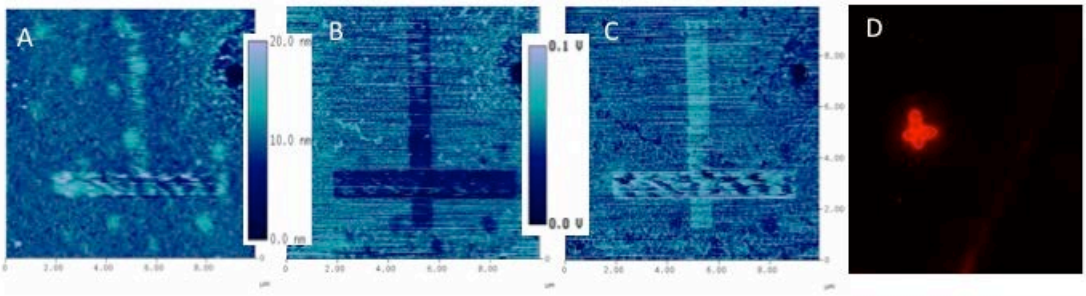

Figure 2: AFM (A-C) and fluorescence images (D) of a pattern of the maleimide dye grafted into the methyl-terminated matrix through coupling to the thiol functional group. The fluorescent dye attaches only on the pattern created with the thiol terminated chemistry.

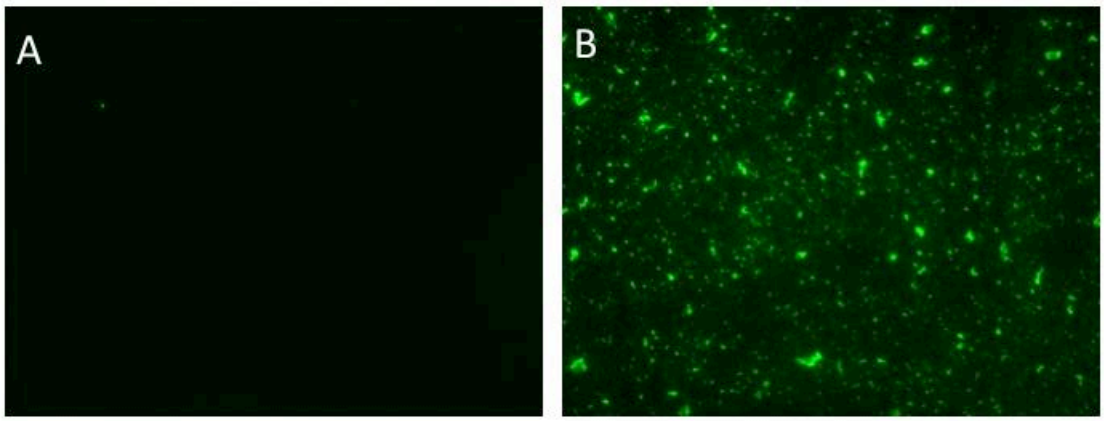

Figure 3: The fluorescently labeled F1 ATPase shows binding to the NTA functionalized surface(B), but very little adsorption on the methyl-terminated surface (A).

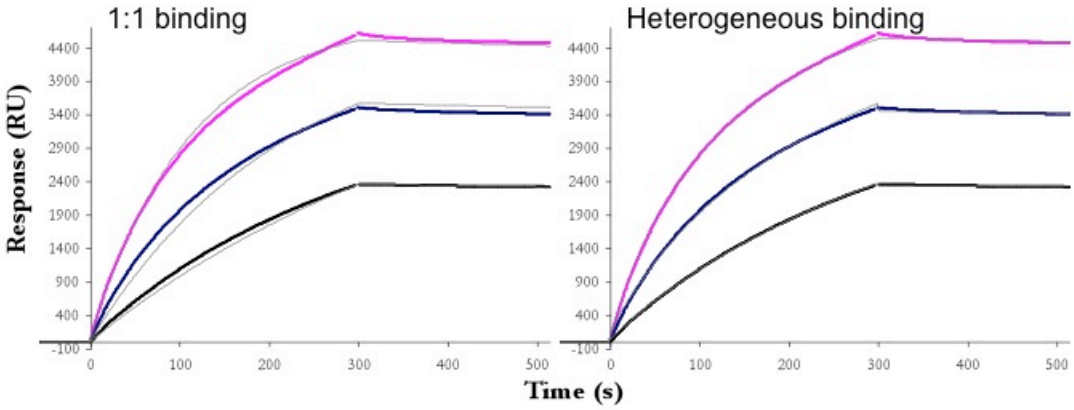

Figure 4: SPR sensorgrams of the adsorption of the F1 ATPase motor on the NTA substrate. Different colors represent different concentrations of protein, and the grey lines are the fits to the data. In order to fit the data, multiple pathways must be included through a heterogeneous binding model. The kinetic rate constants have been determined for binding.

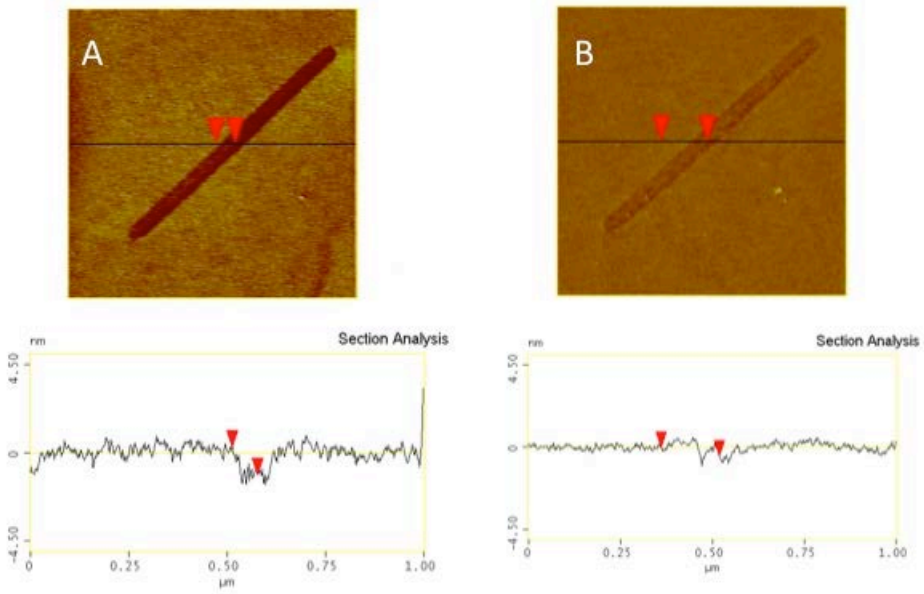

Figure 5: (A) Nanometer scale lines fabricated using anodic lithography to introduce a carboxy-terminated pattern into a methyl terminated matrix. (B) After electroless deposition of copper, the line is much shallower, indicating that the metal has plated selectively in the line.